Kinetics evaluation of CAII–Small compounds using iMSPR-mini/F & HC1000M

iMSPR-mini/F is an entry-level model of icluebio that providing protein-chemical binding analysis quickly and easily. In this application experiment, a binding analysis of CAII with acetazolamide and furosemide, which can be an analytical model for chemical drugs, was performed using HC1000M, a 3D sensor chip. The devices, materials, and conditions used in this experiment can be utilized for various protein-chemical binding analyses.

Materials

- Instrument: iMSPR-mini/F
- Sensor chip: HC1000M
- Immobilization Reagent: Amine coupling kit (ACK50)
- Immobilization buffer: Acetate buffer pH4.0 (AB40)
- Running buffer: 1xHBST (HB50)
- Regeneration buffer: Glycine-HCl pH1.5 (G15)
- Ligand: Carbonic anhydrase II
- Analyte: Acetazolamide (222Da), Furosemide (331 Da)

Procedure

Ligand Immobilization

① Baseline: With inlet tube, 1xHBST is flowed into both channels (ligand channel, reference channel) at a flow rate of 30 ul/min for more than 5 minutes to establish a stable baseline.

② Inject a 1:1 mixture of NHS/EDC into both channels by using the inlet tube at a flow rate of 30 ul/min for 5 minutes followed by 5 minutes of washing in 1xHBST.

③ Inject 50 ug/ml 250ul of CAII (in Acetate buffer pH4.0) only into the ligand channel by using the inlet tube at a flow rate of 10 ul/min for 20 minutes followed by 5 minutes of washing in 1xHBST. (Recommended immobilization level: >5000RU)

④ Inject 200ul of Quenching buffer into both channels by using the inlet tube at a flow rate of 30 ul/min for 5 minutes followed by 5 minutes of washing in 1xHBST.

⑤ Inject 200ul of glycine pH 1.5 into both channels by using the inlet tube at a flow rate of 30 ul/min for 2 minutes followed by 30 minutes or more of washing in 1xHBST to stabilize.

Analyte binding

① Prepare 200ul Acetazolamide and furosemide analyte dilutions into the running buffer (on the same day) to a concentration of 2500, 1250, 625, 313, 156nM.

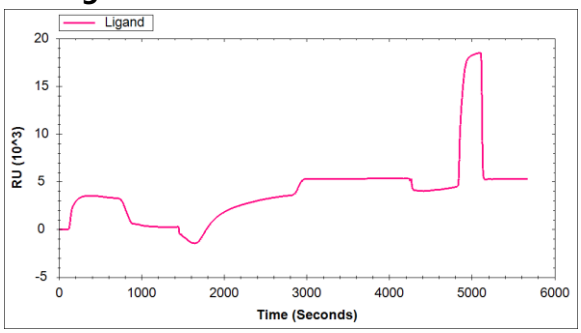
② Introduce 200ul of 2500 nM Acetazolamide at 50 ul/min for a 3 minutes association time followed by 1xHBST for a 7 minute dissociation time.

③ Steps ②-③ were repeated for each concentration.

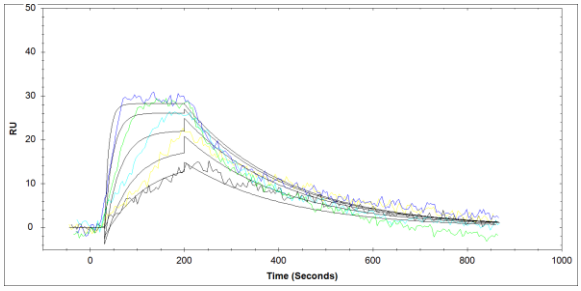
④ Perform Furosemide in the same way.

Results

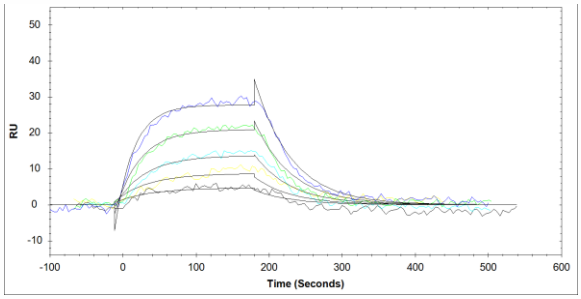
R1. Ligand Immobilization



R2. Acetazolamide Curve fitting: one to one binding model



R3. Curve fitting: one to one binding model



Results summary

S1. Acetazolamide

Contents	Value
Immobilization Level	6000 RU
B_{max}	29.3 RU
K_a (Association rate, 1/M*s)	4.32×10^4
K_d (Dissociation rate, 1/s)	4.68×10^{-4}
K_D (Affinity)	108nM
Chi^2	4.29

S1. Furosemide

Contents	Value
Immobilization Level	6000 RU
B_{max}	69.0 RU
K_a (Association rate, 1/M*s)	8.09×10^3
K_d (Dissociation rate, 1/s)	1.98×10^{-2}
K_D (Affinity)	2450nM
Chi^2	1.83